epo-Fc-CIP-Jan2004.ST25 SEQUENCE LISTING

<110> Sun, Lee-Hwei K Sun, Bill N Sun, Cecily R <120> Fc fusion proteins of human erythropoietin with high biological activities <130> 02SUN2001-A <140> to-be-assigned <141> 2004-01-21 <150> 09/932812 <151> 2001-08-17 <160> 28 <170> PatentIn version 3.1 <210> 1 <211> 29 <212> DNA <213> Artificial Sequence <220> <223> PCR primer <400> 1 cccaagcttg gcgcggagat gggggtgca 29 <210> 2 <211> 27

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agccgag	tcc	tggagaggta	cctcttggag	gccaaggagg	ccgagaatat	cacgacgggc	180					
tgtgctg	jaac	actgcagctt	gaatgagaat	atcactgtcc	cagacaccaa	agttaatttc	240					
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ctgctgt	cgg	aagctgtcct	gcggggccag	gccctgttgg	tcaactcttc	ccagccgtgg	360					
gagcccc	tgc	agctgcatgt	ggataaagcc	gtcagtggcc	ttcgcagcct	caccactctg	420					
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ccactco	gaa	caatcactgc	tgacactttc	cgcaaactct	tccgagtcta	ctccaatttc	540					
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cccaagg	jaca	ccctcatgat	ctcccggacc	cctgaggtca	cgtgcgtggt	ggtggacgtg	780					
agccacg	jaag	accccgaggt	ccagttcaac	tggtacgtgg	acggcgtgga	ggtgcataat	840					
gccaaga	ıcaa	agccacggga	ggagcagttc	aacagcacgt Page		cagcgtcctc	900					

accgttgtgc acc	aggactg gctgaacgg	c aaggagtaca	agtgcaaggt	ctccaacaaa	960
ggcctcccag cct	ccatcga gaaaaccat	c tccaaaacca	aagggcagcc	ccgagaacca	1020
caggtgtaca ccc	tgccccc atcccggga	g gagatgacca	agaaccaggt	cagcctgacc	1080
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ccggagaaca act	acaagac cacacctcc	c atgctggact	ccgacggctc	cttcttcctc	1200
tacagcaagc tca	ccgtgga caagagcag	g tggcagcagg	ggaacgtctt	ctcatgctcc	1260
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<211> 436

<212> PRT

<213> Artificial Sequence

<220>

<223> HuEPO-L-vFc gamma2 with a 27-amino acid leader peptide (Figure 2 A)

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Leu Ser Leu Pro Leu Gly Leu Pro Val Leu Gly Ala Pro Pro Arg Leu 20 25 30

Ile Cys Asp Ser Arg Val Leu Glu Arg Tyr Leu Leu Glu Ala Lys Glu 35 40 45

Ala Glu Asn Ile Thr Thr Gly Cys Ala Glu His Cys Ser Leu Asn Glu 50 60

Asn Ile Thr Val Pro Asp Thr Lys Val Asn Phe Tyr Ala Trp Lys Arg 65 70 75 80

Met Glu Val Gly Gln Gln Ala Val Glu Val Trp Gln Gly Leu Ala Leu 85 90 95

Leu Ser Glu Ala Val Leu Arg Gly Gln Ala Leu Leu Val Asn Ser Ser 100 105 110

epo-Fc-CIP-Jan2004.ST25 Gln Pro Trp Glu Pro Leu Gln Leu His Val Asp Lys Ala Val Ser Gly 115 120 Leu Arg Ser Leu Thr Thr Leu Leu Arg Ala Leu Gly Ala Gln Lys Glu 130 140 Ala Ile Ser Pro Pro Asp Ala Ala Ser Ala Ala Pro Leu Arg Thr Ile 145 150 155 160 Thr Ala Asp Thr Phe Arg Lys Leu Phe Arg Val Tyr Ser Asn Phe Leu 165 170 175 Arg Gly Lys Leu Lys Leu Tyr Thr Gly Glu Ala Cys Arg Thr Gly Asp 180 185 190 Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val 210 215 220 Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu 225 230 235 240 Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Asp Val Ser 245 250 255 His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu 260 270 Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr 275 280 285 Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn 290 295 300 Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Ser 305 310 315 Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln 325 330 335 Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val 340 345 350 Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val 355 360 365

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro 370 380 Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr 385 390 395 400 Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val 405 410 415 Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu 420 430 Ser Pro Gly Lys 435 <210> 19 <211> 1335 <212> DNA <213> Artificial Sequence <220> <223> HuEPO-L-vFc gamma4 (Figure 2B) <400> aagcttggcg cggagatggg ggtgcacgaa tgtcctgcct ggctgtggct tctcctqtcc 60 ctgctgtcgc tccctctggg cctcccagtc ctgggcgccc caccacgcct catctgtgac 120 agccgagtcc tggagaggta cctcttggag gccaaggagg ccgagaatat cacgacgggc 180 tgtgctgaac actgcagctt gaatgagaat atcactgtcc cagacaccaa agttaatttc 240 tatgcctgga agaggatgga ggtcgggcag caggccgtag aagtctggca gggcctggcc 300 ctgctgtcgg aagctgtcct gcggggccag gccctgttgg tcaactcttc ccaqccqtqq 360 gagcccctgc agctgcatgt ggataaagcc gtcagtggcc ttcgcagcct caccactctg 420 cttcgggctc tgggagccca gaaggaagcc atctccctc cagatgcggc ctcagctgct 480 ccactccgaa caatcactgc tgacactttc cgcaaactct tccgagtcta ctccaatttc 540 ctccggggaa agctgaagct gtacacaggg gaggcctgca ggacagggga cggatccggt 600 ggcggttccg gtggaggcgg aagcggcggt ggaggatcag agtccaaata tggtcccca 660 tgcccaccat gcccagcacc tgagttcgcg gggggaccat cagtcttcct gttccccca 720 aaacccaagg acactctcat gatctcccgg acccctgagg tcacgtgcgt ggtggtggac 780

gtgagccagg aagaccccga ggtccagttc aactggtacg tggatggcgt ggaggtgcat

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840

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ctcaccgtcc	tgcaccagga	ctggctgaac	ggcaaggagt	acaagtgcaa	ggtctccaac	960
aaaggcctcc	cgtcctccat	cgagaaaacc	atctccaaag	ccaaagggca	gccccgagag	1020
ccacaggtgt	acaccctgcc	cccatcccag	gaggagatga	ccaagaacca	ggtcagcctg	1080
acctgcctgg	tcaaaggctt	ctaccccagc	gacatcgccg	tggagtggga	gagcaatggg	1140
cagccggaga	acaactacaa	gaccacgcct	cccgtgctgg	actccgacgg	ctccttcttc	1200
ctctacagca	ggctaaccgt	ggacaagagc	aggtggcagg	aggggaatgt	cttctcatgc	1260
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<213> Artificial Sequence

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<223> HuEPO-L-vFc gamma4 with a 27-amino acid leader peptide (Figure 2B)

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Leu Ser Leu Pro Leu Gly Leu Pro Val Leu Gly Ala Pro Pro Arg Leu 20 25 30

Ile Cys Asp Ser Arg Val Leu Glu Arg Tyr Leu Leu Glu Ala Lys Glu 35 40 45

Ala Glu Asn Ile Thr Thr Gly Cys Ala Glu His Cys Ser Leu Asn Glu 50 55 60

Asn Ile Thr Val Pro Asp Thr Lys Val Asn Phe Tyr Ala Trp Lys Arg 75 80

Met Glu Val Gly Gln Gln Ala Val Glu Val Trp Gln Gly Leu Ala Leu 85 90 95

Leu Ser Glu Ala Val Leu Arg Gly Gln Ala Leu Leu Val Asn Ser Ser 100 105 110 Page 10

Gln Pro Trp Glu Pro Leu Gln Leu His Val Asp Lys Ala Val Ser Gly 115 120 125 Leu Arg Ser Leu Thr Thr Leu Leu Arg Ala Leu Gly Ala Gln Lys Glu 130 135 140 Ala Ile Ser Pro Pro Asp Ala Ala Ser Ala Ala Pro Leu Arg Thr Ile 145 150 155 160 Thr Ala Asp Thr Phe Arg Lys Leu Phe Arg Val Tyr Ser Asn Phe Leu 165 170 175 Arg Gly Lys Leu Lys Leu Tyr Thr Gly Glu Ala Cys Arg Thr Gly Asp 180 185 190 Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe 210 215 220 Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr 225 230 235 240 Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Asp Val 245 250 255 Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val 260 265 270 Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser 275 280 285 Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu . 290 295 300 Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser 305 310 315 320 Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro 325 330 335 Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln 340 350 Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Page 11

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr 370 380

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu 385 390 395 400

Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser 405 410 415

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser 420 425 430

Leu Ser Leu Gly Lys 435

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<212> DNA

<213> Artificial Sequence

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<223> HuEPO-L-vFc gamma1 (Figure 2C)

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Page 12

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aagacaaagc cgo	egggagga	gcagtacaac	agcacgtacc	gggtggtcag	cgtcctcacc	900
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ctggtcaaag gct	tctatcc	cagcgacatc	gccgtggagt	gggagagcaa	tgggcagccg	1140
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<210> 22

<211> 435

<212> PRT

<213> Artificial Sequence

<220>

<223> HuEPO-L-vFc gammal with a 27-amino acid leader peptide (Figure 2C)

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Leu Ser Leu Pro Leu Gly Leu Pro Val Leu Gly Ala Pro Pro Arg Leu 20 25 30

Ile Cys Asp Ser Arg Val Leu Glu Arg Tyr Leu Leu Glu Ala Lys Glu 35 40 45

Ala Glu Asn Ile Thr Thr Gly Cys Ala Glu His Cys Ser Leu Asn Glu 50 55 60

Asn Ile Thr Val Pro Asp Thr Lys Val Asn Phe Tyr Ala Trp Lys Arg 65 70 75 80

Met Glu Val Gly Gln Gln Ala Val Glu Val Trp Gln Gly Leu Ala Leu 85 90 95

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Gln	Pro	Trp 115	Glu	Pro	Leu	Gln	Leu 120	His	٧a٦	Asp	Lys	Ala 125	val	Ser	Gly
Leu	Arg 130	Ser	Leu	Thr	Thr	Leu 135	Leu	Arg	Ala	Leu	Gly 140	Ala	Gln	Lys	Glu
Ala 145	Ile	Ser	Pro	Pro	Asp 150	Ala	Ala	Ser	Ala	Ala 155	Pro	Leu	Arg	Thr	11e 160
Thr	Ala	Asp	Thr	Phe 165	Arg	Lys	Leu	Phe	Arg 170	Val	Tyr	Ser	Asn	Phe 175	Leu
Arg	Gly	Lys	Leu 180	Lys	Leu	Tyr	Thr	Gly 185	Glu	Ala	Cys	Arg	Thr 190	Gly	Asp
Gly	Ser	Gly 195	Gly	Gly	Ser	Gly	G]y 200	Gly	Gly	Ser	Gly	Gly 205	Gly	Gly	Ser
Asp	Lys 210	Thr	His	Thr	Cys	Pro 215	Pro	Cys	Pro	Ala	Pro 220	Glu	٧a٦	Ala	Gly
Gly 225	Pro	Ser	val	Phe	Leu 230	Phe	Pro	Pro	Lys	Pro 235	Lys	Asp	Thr	Leu	Met 240
Ile	Ser	Arg	Thr	Pro 245	Glu	∨al	Thr	Cys	va1 250	Val	٧al	Asp	val	Ser 255	нis
Glu	Asp	Pro	G1u 260	val	Lys	Phe	Asn	Trp 265	Tyr	val	Asp	Gly	Val 270	Glu	٧al
ніѕ	Asn	Ala 275	Lys	Thr	Lys	Pro	Arg 280	Glu	Glu	Gln	Tyr	Asn 285	Ser	Thr	Tyr
Arg	Val 290	val	Ser	val	Leu	Thr 295	val	Leu	His	Gln	Asp 300	Trp	Leu	Asn	Gly
Lys 305	Glu	Tyr	Lys	Cys	Lys 310	val	Ser	Asn	Lys	Ala 315	Leu	Pro	Ala	Ser	Ile 320
Glu	Lys	Thr	Ile	Ser 325	Lys	Ala	Lys	Gly	G]n 330	Pro	Arg	Glu	Pro	G]n 335	۷al
Tyr	Thr	Leu	Pro 340	Pro	Ser	Arg	Asp	G1u 345	Leu	Thr	Lys	Asn	G1n 350	٧a٦	Ser

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu 355 360 365

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro 370 375 380

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val 385 390 395 400

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met 405 410 415

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser 420 425 430

Pro Gly Lys 435

<210> 23

<211> 16

<212> PRT

<213> Artificial sequence, 16-amino acid peptide linker

<400> 23

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<210> 24

<211> 15

<212> PRT

<213> Human IgG1 hinge sequence

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<210> 25

<211> 10

<212> PRT

<400> 25

Asp Lys Thr His Thr Cys Pro Pro Cys Pro 1 5 10

<210> 26

<211> 232

<212> PRT

<213> Human IgG1 Fc with native hinge, CH2 and CH3 domains

<400> 26

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Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val 35 40 45

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val 50 60

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln 65 70 75 80

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln 85 90 95

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala 100 105 110

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro 115 120 125

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr 130 135 140

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser 145 150 155 160

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Page 16 Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr 180 185 190

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe 195 200 205

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys 210 220

Ser Leu Ser Leu Ser Pro Gly Lys 225 230

<210> 27

<211> 228

<212> PRT

<213> Human IgG2 Fc with native hinge, CH2 and CH3 domains

<400> 27

Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$

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Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Asp Val Ser 35 40 45

His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu 50 55 60

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr 65 70 75 80

Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn 85 90 95

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro 100 105 110

Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln
115 120 125

epo-Fc-CIP-Jan2004.ST25 Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val 130 135 140 Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val 145 150 155 160 Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr 180 185 190 Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val 195 200 205 Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu 210 220 Ser Pro Gly Lys <210> 28 229 <211> <212> PRT Human IgG4 Fc with native hinge, CH2 and CH3 domains <400> 28 Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro Ala Pro Glu Phe 1 5 10 15 Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr 20 25 30 Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Asp Val 35 40 45 Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val 50 55 60 Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser 65 70 75 80

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu 85 90 95

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser 100 105 110

Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro 115 120 125

Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln 130 135

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala 145 150 155 160

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr 165 170 175

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu 180 185 190

Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser 195 200 205

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser 210 215 220

Leu Ser Leu Gly Lys 225